Oxytetracycline (Terramycin[7] 200 for Fish) Medicated Feed Clinical Field Trials - INAD 9332

Year 2012 - 2014 Annual Summary Report on the Use of Oxytetracycline (Terramycin[7] 200 for Fish) Medicated Feed in Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

Oxytetracycline (Terramycin[7] 200 for Fish) medicated feed has been used effectively in the U. S. under compassionate INAD Exemption #9332 to either: (1) control/prevent mortality in a variety of fish caused by common fish bacterial pathogens, or (2) for marking skeletal tissue of early life stages of fish. In calendar years 2012 - 2014 (CY12-14) the efficacy of oxytetracycline (Terramycin[7] 200 for Fish) medicated feed (OTF) was evaluated in 84 trials involving approximately 9.3 million fish to control mortality in a variety of test fish caused by a variety of infectious fish pathogens or to apply a skeletal mark to fish. Trials were conducted at 16 fish culture facilities, including one U.S. Fish and Wildlife Service fish hatchery, 13 state hatcheries, one tribal hatchery, and one private fish culture hatchery. The compassionate study protocol under which treatments were administered allowed the investigator to use OTF at a dosage of either: 1) 2.5 - 3.75 g drug/100 lbs fish/d for 10 days; or 2) 10 g drug/100 lbs fish/d for 14 days. Overall, results of trials conducted in CY12-14 indicated that treatments appeared to be efficacious in

approximately 82% of the trials, ineffective in 5% of the trials, and characterized as inconclusive in 13% of the trials.

Introduction

The current labels for OTF use in aquaculture limits use to: Salmonids - 1) control of ulcer disease (*Hemophilus piscium*); 2) the control of furunculosis (*Aeromonas salmonicida*); 3) control of bacterial hemorragic septicemia (*A. Liquefaciens*); and 4) pseudomonas disease (*Pseudomonas* spp.). Dosing: 2.5 - 3.75 g per 100 lbs fish per day for 10 days. Freshwater-reared salmonids - control of coldwater disease (*Flavobacterium psychrophilum*). Dosing: 3.75 g per 100 lbs fish per day for 10 days. All freshwater-reared *Oncorhynchus mykiss* - control columnaris diesase (*Flavobacterium columnare*). Dosing: 3.75 g per 100 lbs fish per day for 10 days. Pacific salmon - to mark skeletal tissue. Dosing: 250 mg/kg fish/day for 4 days in salmon less then 30 g . Catfish - control of bacterial hemorrhagic septicemia (*Aeromonas liquefaciens*) and pseudomonas disease (*Pseudomonas* spp.). Dose: 2.5 - 3.75 g per 100 lbs fish per day for 10 days. These label restrictions limit the overall utility of approved OTF use in aquaculture.

Historically, OTF treatments have been used by fish culturists to control mortality in salmonids caused by bacterial coldwater disease (CWD; causative agent *Flavobacterium psychrophilus*) and columnaris (causative agent *F. columnare*). Fish culturists and fish health professionals have also found that OTF is effective therapy to control mortality in fishes caused by enteric redmouth (causative agent *Yersinia ruckeri*), vibriosis (causative agent various members of the genus *Vibrio*), and other less common bacterial diseases. However, at this time, OTF has a limited label for such uses, and the only legal way to use OTF for such non-approved uses is through an INAD.

Fish culturists have also reported that oxytetracycline treatment is a useful tool for marking the skeletal tissue in salmonid fish when treated at a size in which fish body weight does not exceed 2 g. Marks were visible on skeletal tissue of fish immediately after the treatment period, and had still been visible for several months afterwards. In addition, studies have been conducted in which different oxytetracycline drug dosages were used to mark skeletal tissue of test fish. Summary conclusions from such studies indicated that not only did various dosages of oxytetracycline effectively mark skeletal tissue, but there were also no evidence of any toxic or adverse effects to the fish.

The proposed treatment strategy (i.e., dosage and duration) for the use of OTF in fish is designed to meet the needs of individual fish species, individual fish lots, and a variety of environmental conditions. In all cases, treatment goals are to (1) minimize the negative effects of disease on fish health, quality, and survival, and (2) help meet fishery management objectives. Because many factors can affect the success or failure of oxytetracycline medicated feed therapy, supplemental efficacy data from compassionate Investigational New Animal Drug (INAD) use, as well as efficacy data from controlled, replicated studies that are scientifically valid and statistically defensible (i.e., pivotal), are needed to gain approval of OTF use in aquaculture.

Purpose of Report

The purpose of this report is to summarize the results of CY12-14 OTF field efficacy trials conducted under INAD #9332. Furthermore, it is expected that data from these trials will be used

to enhance the existing OTF database that has been established from studies conducted in previous years for the purpose of expanding the approved label for OTF.

Facilities, Materials, and Treatment Procedures

1. Facilities

A total of 84 trials were conducted at 16 fish culture facilities, including one U.S. Fish and Wildlife Service fish hatchery, 13 state fish hatcheries, one tribal hatchery, and one private fish culture hatchery. Water temperature during treatments at the various testing facilities ranged from 41.50 – 85.9 EF, with a mean treatment temperature of 64.1EF.

2. Test article used

The OTF used in CY12-14 efficacy trials was Terramycin[7] 200 which contained 200 g active oxytetracycline (from oxytetracycline dihydrate) per pound of Type A Medicated Article. All Terramycin[7] 200 was supplied by Phibro Animal Health, 75 Challenger Road Ridgefield Park, NJ. OTF was prepared with Phibro brand product by one of several commercial fish feed manufacturers (e.g.,Skretting, Rangen Inc.) or by top-coating feed at the testing site by the investigator, monitor, or their designee.

3. Treatment regimen

As described in the Study Protocol, Investigators were allowed to use OTF either within the current label range of 2.5 - 3.75 g of active drug/100 lbs of fish/d for 10 days (approximately 46% of trials were conducted using this treatment regimen); or 10.0 g of

active drug/100 lbs of fish/d for 14 days (approximately 42% of trials were conducted using this treatment regimen). Note: the online INAD database rounds the 3.75 g of active drug/100 lbs of fish/d number to 3.8 g of active drug/100 lbs of fish/d and this is how it is reported on all of the data forms.

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 12% of trials) deviated from the protocol. The following are the explanations for the deviations in each of the trials. 1) In one trial, fish were fed at 8.0 g drug/100 lbs fish/d for 14 days. This deviation occurred because of a miscalculation in the percent premix. 2) In two trials, fish were fed at 8.3 and 9.3 g drug/100 lbs fish/d for 14 days. These deviations occurred because fish were slightly bigger than predicted at the start of the treatments. 3) In one trial, fish were fed at 10 g drug/100 lbs fish/d for 26 days. This deviation occurred because the investigator did back to back treatments so the fish would not relapse once they were transitioned to a minnow diet. The investigator was contacted and told to only treat for 14 days for future studies. 4) In two trials, fish were fed at 3.8 g drug/100 lbs fish/d for 7 days. These deviations occurred because the fish were moved to other parts of the hatchery. The investigator was contacted and told that a full 10 days of treatment is needed for future studies. 5) In two trials, fish were fed at 3.8 g drug/100 lbs fish/d for 13 - 16 days. These deviations occurred because the investigator had wanted to use up all of the medicated feed. The investigator was contacted and told that treatments can't exceed 10 days for any future studies. 6) In one trial, fish were fed at 3.8 g drug/100 lbs fish/d for 20 days. This deviation occurred because the disease had returned after 5 post-treatment days and another 10 day treatment was initiated. The investigator was

contacted and advised that there needed to be at least 10 days between treatments. 7) In one trial, fish were fed at 10 g drug/100 lbs fish/d for 4 days. This deviation occurred because the investigator's fish health center advised that there was not a significant amount of mortality to continue the treatment.

Fish Species and Fish Diseases Involved in CY12-14 Trials

1. Species of fish treated

10 fish species, including five salmonids and five non-salmonids were treated during CY12-14. Treated fish ranged in length from 1.0 - 48.0 in. and the average length of all treated fish was 4.7 in. Fish species treated included:

Salmonids:

brook trout (*Salvelinus fontinalis*)

Chinook salmon (*Oncorhynchus tshawytscha*)

coho salmon (*O. kisutch*)

cutthroat trout (*O.clarki*)

rainbow trout (*O. mykiss*)

Non-salmonids:

American shad (*Alosa sapidissima*)

muskellunge (*Esox masquinongy*)

northern pike (*E. lucius*)

walleye (*Sander vitreus*)

white sturgeon (*Acipenser transmontanus*)

2. Disease/Purpose treated

Test fish were either treated with OTF to 1) provide a readable mark on skeletal tissue; or 2) treated to either control/prevent mortality caused by the following diseases during CY12-14: Aeromonas Hydrophila; Aeromonas spp.; Bacterial Hemorrhagic Septicemia; Columnaris; Bacterial Coldwater Disease; Flavobacteriosis; General Systemic Bacterial Infection; Motile Aeromonad; and Motile Aeromonad Septicemia.

Data Collected

1. Pathologist's reports

A pathologist=s report was submitted for 2% of the studies. Pathology reports are important for accurate interpretation of study results because they typically contain the following information:

A. Description of how the identity of disease agent(s) was verified,

B. Disease identification records that confirm the presence of the disease agent,

C. The name and title of the individual performing the diagnosis.

Additionally, evidence would typically be provided to document that there were no secondary infections or infestations caused by unrelated disease agents in the population of

test fish. As a result, pathology reports provide essential information if efforts are to expand/extend an existing approved label.

2. Treatment response and drug accountability data

Drug receipt reports, drug use reports, diagnosis, treatment, and mortality reports (including adverse effects/toxicity observations), and fish disposition reports were prepared by study investigators through the online INAD database. Such reports were routed through the study monitor for review, and then sent to the AADAP Office for review, data analysis and report writing, and archiving in permanent files.

As stated in the Study Protocol, mortality data was to be collected for at least five days prior to treatment, during treatment, and for at least 10 d post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis. However, for a variety of reasons, not all requested mortality data was collected. Reasons for an incomplete mortality record include: 1) splitting fish into additional rearing units to ease crowding and improve culture conditions, and 2) stocking early life stage fish shortly after final treatment.

Discussion of Study Results:

1. Relevance of study to expanding current label claim for OTF

Results of CY12-14 trials conducted under Compassionate INAD exemption #9332 are similar to results detailed in reports previously submitted to FDA under INAD=s #9332 and #9006.

2. General observations on the efficacy of OTF for the control of bacterial diseases in fish species or to apply a skeletal mark (Note: Table 1 provides a summary of all trials characterized as effective; Table 2 provides a summary of all trials characterized as ineffective; Table 3 provides a summary of all trials characterized as inconclusive; and Table 4 provides summary data for all trials conducted during CY12-14 under INAD #9332).

A. Efficacy at 2.50 - 3.8 g/100 lbs fish/d for 7 - 20 days

American shad, brook trout, cutthroat trout, rainbow trout, walleye, and white sturgeon were treated with 2.5 - 3.8 g OTF/100 lbs of fish/d for 7 - 20 days in 44 trials (Tables 1 - 3). Investigators used OTF to either apply a skeletal mark or to control mortality caused by columnaris, general systemic bacterial infection, or motile aeromonad. OTF treatments appeared effective in 37 trials, ineffective in two trials, and characterized as inconclusive in five trials.

B. Efficacy at 8 - 10 g/100 lbs fish/d for 4 - 26 days

Chinook salmon, coho salmon, cutthroat trout, muskellunge, northern pike, rainbow trout, and walleye were treated with 8 - 10 g OTF/100 lbs of fish/d for 4 - 26 days in 40 trials (Tables 1 - 3). Investigators used OTF to control mortality caused by *Aeromonas Hydrophila*, *Aeromonas spp*., *Bacterial Hemorrhagic Septicemia*, columnaris, bacterial coldwater disease, flavobacteriosis, and *Motile Aeromonad Septicemia*. OTF treatments appeared effective in 32 trials, ineffective in two trials, and was characterized as inconclusive in six trials.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY12-14.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for Oxytetracycline (Terramycin[7] 200 for Fish) INAD #9332

No changes have occurred to the current study protocol for Oxytetracycline (Terramycin[7] 200 for Fish) INAD #9332.

Facility Sign-up List

Please see ATable 5. Facilities and Names of Investigators@ for facilities that signed-up to participate in the Oxytetracycline (Terramycin[7] 200 for Fish) INAD #9332 during

CY12-14. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facility had Oxytetracycline (Terramycin[7] 200 for Fish) medicated feed or premix on-hand during CY12-14 but never used the drug:

1. Keahole Point Fish LLC

Correspondence sent to Oxytetracycline (Terramycin[7] 200 for Fish) Participants

Please see the attached correspondence that was sent to all Oxytetracycline (Terramycin[7] 200 for Fish) participants after the AADAP Office received their sign-up form for CY12-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY12-14 was 9,342,578. The total number of treated fish to count against the Oxytetracycline (Terramycin[7] 200 for Fish) treatment use authorization dated June 25, 2007 is 38,180,781.

Summary of Study Results

Oxytetracycline (Terramycin[7] 200 for Fish) medicated feed was used at dosages ranging from 2.5 - 10.0 g active drug/100 lbs fish/d in 84 treatment trials. Treatment durations ranged from 4 - 26 days. Treatment trials involved 10 different fish species and approximately 9.3 million fish.

Treated fish ranged in length from 1.0 - 48.0 in. Water temperature during treatment ranged from 41.5 – 85.9 EF, with a mean treatment temperature of 64.1EF. Overall results showed that treatment in approximately 82% of trials appeared to be efficacious, while 5% appeared ineffective, and 13% were characterized as inconclusive. No evidence of toxicity or adverse effects related to OTF treatment was reported in any of the trials. However, based on a general lack of untreated control fish, replication, randomization, etc., it is understood that these data will only be considered as supportive or ancillary data. None-the-less, the data described above should provide useful corroborative data to support a future expanded label claim for OTF for these disease/marking indications. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #9332. In future trials conducted under this INAD, efforts will continue to be directed towards the generation of high quality data.

References

Warren, J.W. 1991. Diseases of hatchery fish. U.S. Fish and Wildlife Service, Portland, Oregon, 92 p.

Table 1. Summary of CY 2012 - 2014 OTF Treatment Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease/Purpose	Dose (g/100 lbs)	Number of Treatment Days	Temp. (^{o}F)
Matapeake	4	American Shad	2.0	450,000	Marking	3.8	10	80.0 – 85.1
Giant Springs SFH	2	Brook Trout	2.4 – 3.2	125,367	Marking	3.8	10 - 16	54.0
Yellowstone River Trout SFH	1	Cutthroat Trout	2.9	42,000	Marking	3.8	10	52.0
Bluewater SFH	12	Rainbow Trout	2.9 – 4.5	904,000	Marking	3.8	10	58.0 – 59.0
Giant Springs SFH	3	Rainbow Trout	2.6 – 5.6	329,981	Marking	3.8	10 - 13	54.0
Rathbun SFH	3	Walleye	7.3 – 7.5	69,315	Columnaris	3.8	10	83.4 – 85.9
Rathbun SFH	5	Walleye	6.5 – 7.5	105,539	General Systemic Bacterial Infection	3.8	10	77.9 – 84.0
Rathbun SFH	4	Walleye	1.9 – 5.8	106,499	Motile Aeromonad	3.8	10	75.2 – 77.3
Sterling Caviar	3	White Sturgeon	7.9 – 12.0	33,692	General Systemic Bacterial Infection	3.0 – 3.8	10	67.3 – 69.6
Coleman NFH	7	Chinook Salmon	3.1 – 5.1	2,437,127	Columnaris	8.0 – 10.0	14	64.0 – 69.2
Dexter Ponds	2	Chinook Salmon	3.0 – 5.5	709,000	Columnaris	10.0	14	60.0 – 61.0
Dworshak NFH - Tribal Portion	1	Coho Salmon	4.5	332,247	Bacterial Coldwater Disease	10.0	14	41.5
Murray Springs SFH	5	Cutthroat Trout	1.0 – 1.1	301,003	Bacterial Coldwater Disease	10.0	14	52.0
Washoe Park Trout SFH	1	Cutthroat Trout	1.0	300,000	Bacterial Coldwater Disease	10.0	14	56.0
Spirit Lake SFH	2	Muskellunge	3.4 – 4.2	90,111	Aeromonas Hydrophila	10.0	14 - 26	69.5 – 72.5

Table 1. Summary of CY 2012 - 2014 OTF Treatment Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease/Purpose	Dose (g/100 lbs)	Number of Treatment Days	Temp. (^{o}F)
Spirit Lake SFH	2	Northern Pike	2.0 – 2.5	559,978	Aeromonas Hydrophila	10.0	14	57.0 – 60.9
American Fall SFH	1	Rainbow Trout	3.5	55,000	Bacterial Coldwater Disease	10.0	14	56.0
Boulder Rearing Station	2	Rainbow Trout	4.5 – 19.3	70,585	Bacterial Hemorrhagic Septicemia	10.0	14	52.0
Boulder Rearing Station	3	Rainbow Trout	2.4 – 3.2	173,700	Bacterial Coldwater Disease	10.0	14	52.0 – 53.0
Boulder Rearing Station	1	Rainbow Trout	19.5	2,700	Motile Aeromonad Septicemia	10.0	14	52.0
Clarks Fork SFH	2	Rainbow Trout	2.2 – 2.7	88,700	Bacterial Coldwater Disease	10.0	14	52.0
Rathbun SFH	1	Walleye	2.5	27,603	Aeromonas spp.	10.0	14	78.0
Spirit Lake SFH	2	Walleye	2.7 – 3.9	79,736	Aeromonas Hydrophila	10.0	14	71.5 – 72.3

Table 2. Summary of CY 2012 - 2014 OTF Treatment Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (g/100 lbs)	Number of Treatment Days	Temp. (^{o}F)
Matapeake	1	American Shad	2.0	200,000	Marking	3.8	10	83.1
Sterling Caviar	1	White Sturgeon	48.0	825	General Systemic Bacterial Infection	2.5	10	64.6
Murray Springs SFH	1	Cutthroat Trout	1.0	121,539	Bacterial Coldwater Disease	10.0	14	52.0
Boulder Rearing Station	1	Rainbow Trout	3.1	83,300	Bacterial Hemorrhagic Septicemia	10.0	14	52.0

Table 3. Summary of CY 2012 - 2014 OTF Treatment Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (g/100 lbs)	Number of Treatment Days	Temp. (^{o}F)
Murray Springs SFH	1	Rainbow Trout	2.6	5,000	Marking	3.8	10	52.0
Rathbun SFH	3	Walleye	2.7 – 5.3	43,949	General Systemic Bacterial Infection	3.8	7 - 20	75.2 – 79.0
Rathbun SFH	1	Walleye	3.1	21,509	Motile Aeromonad	3.8	7	75.6
Coleman NFH	2	Chinook Salmon	3.2 – 5.1	1,097,367	Columnaris	10.0	4 - 14	64.0 – 68.5
Murray Springs SFH	1	Cutthroat Trout	1.1	81,906	Bacterial Coldwater Disease	10.0	14	52.0
Big Springs Trout	1	Rainbow Trout	2.0	122,700	Bacterial Coldwater Disease	10.0	14	57.0
Big Springs Trout	2	Rainbow Trout	5.4 – 8.8	170,600	Flavobacteriosis	8.3 – 9.3	14	52.0

Table 4. Summary Data Regarding Summary of CY 2012 - 2014 OTF Treatment Trials

Total Fish Treated:	9,342,578
Number of fish treated in efficacious trials	7,393,883
Number of fish treated in ineffective trials	405,664
Number of fish treated in inconclusive trials	1,543,031
Total number of trials:	**84**
Efficacious trials	69
Ineffective trials	4
Inconclusive trials	11
Treatment Regimens Used:	
2.5 - 3.8 g/100 lbs fish/day for 7 - 20 days	44 trials
8.0 – 9.3 g/100 lbs fish/day for 14 days	3 trials
10.0 g/100 lbs fish/day for 4 - 26 days	37 trials
Treatment Water Temperature (EF):	
Temperature Range	41.5 – 85.9
Average Temperature	64.1
Size of Treated Fish (in.):	
Size Range	1.0 - 48.0
Average Length	4.7

Species Treated:

Salmonids:

brook trout (*Salvelinus fontinalis*)
Chinook salmon (*Oncorhynchus tshawytscha*)
coho salmon (*O. kisutch*)
cutthroat trout (*O.clarki*)
rainbow trout (*O. mykiss*)

Non-salmonids:

American shad (*Alosa sapidissima*)
muskellunge (*Esox masquinongy*)
northern pike (*E. lucius*)
walleye (*Sander vitreus*)
white sturgeon (*Acipenser transmontanus*)